=> fil medl,caplus,biosis,embase,wpids;s angiogenes? associat? protein and n termin?

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	110.52	110.67

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-5.01	-5.01

Harris 332063

FILE 'MEDLINE' ENTERED AT 13:28:45 ON 24 JAN 2000

FILE 'CAPLUS' ENTERED AT 13:28:45 ON 24 JAN 2000
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.

FILE 'BIOSIS' ENTERED AT 13:28:45 ON 24 JAN 2000

FILE 'EMBASE' ENTERED AT 13:28:45 ON 24 JAN 2000

FILE 'WPIDS' ENTERED AT 13:28:45 ON 24 JAN 2000

L1 0 FILE MEDLINE
L2 0 FILE CAPLUS
L3 0 FILE BIOSIS
L4 0 FILE EMBASE
L5 0 FILE WPIDS

TOTAL FOR ALL FILES
L6 0 ANGIOGENES? ASSOCIAT? PROTEIN AND N TERMIN?

=> s angiogenes? and plasminogen?

```
L7          337 FILE MEDLINE
L8          386 FILE CAPLUS
L9          358 FILE BIOSIS
L10         389 FILE EMBASE
L11          36 FILE WPIDS

TOTAL FOR ALL FILES
L12        1506 ANGIOGENES? AND PLASMINOGEN?

=> s l12 and n termin?

L13           7 FILE MEDLINE
L14          16 FILE CAPLUS
L15           6 FILE BIOSIS
L16          10 FILE EMBASE
L17           3 FILE WPIDS

TOTAL FOR ALL FILES
L18          42 L12 AND N TERMIN?

=> s kringle domain and l18

L19           1 FILE MEDLINE
L20           1 FILE CAPLUS
L21           1 FILE BIOSIS
L22           2 FILE EMBASE
L23           1 FILE WPIDS

TOTAL FOR ALL FILES
L24           6 KRINGLE DOMAIN AND L18

=> dup rem l24

PROCESSING COMPLETED FOR L24
L25           3 DUP REM L24 (3 DUPLICATES REMOVED)

=> d cbib abs 1-3;s l18 not l24


L25  ANSWER 1 OF 3  WPIDS COPYRIGHT 2000   DERWENT INFORMATION LTD
AN   1999-430389 [36]   WPIDS
AB   WO   9935248 A UPAB: 19990908
     NOVELTY - Production of angiostatin (I) by solubilizing and refolding
     recombinant protein is new.
          DETAILED DESCRIPTION - Production of angiostatin (I) comprises:
          (a) culturing cells expressing a gene (II) for (I);
          (b) recovering the gene product;
          (c) solubilizing then refolding it at high pH, and
          (d) isolating the properly folded (I).
          INDEPENDENT CLAIMS are also included for the following:
          (1) similar process without the solubilization step; and
          (2) polypeptides (IIa) encoded by any of 20 specified polynucleotide
     sequences (A) (all are given in the specification).
          ACTIVITY - Anticancer; anti-angiogenic.
          MECHANISM OF ACTION - (I) inhibits the growth and migration of
     endothelial cells, and their ability to form tubular structures. Purified
     recombinant protein comprising kringle domains 1-3 of
     human plasminogen, produced in E. coli, was tested at 20 mu g/ml
     for inhibition of human microvascular endothelial cells in a transwell
     apparatus, using vascular endothelial growth factor as the
     chemoattractant. The protein reduced the number of migrating cells by
```

about 50% compared to the case with chemoattractant only.
USE - (I) is used to inhibit growth of tumors and **angiogenesis**.
ADVANTAGE - This method provides efficient solubilization of (I)-containing bacterial inclusion bodies and subsequent refolding to biologically active protein.
Dwg.0/6

L25 ANSWER 2 OF 3 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
1999161568 EMBASE Diurnal variations in angiostatin in human tear fluid: A possible role in prevention of corneal neovascularization. Sack R.A.; Beaton A.R.; Sathe S.. R.A. Sack, SUNY College of Optometry, 100 East 24 St., Manhattan, NY 10010, United States. RSack25968@AOL.com. Current Eye Research 18/3 (186-193) 1999.
Refs: 31.
ISSN: 0271-3683. CODEN: CEYRDM. Pub. Country: Netherlands. Language: English. Summary Language: English.
AB Purpose. Although overnight eye closure is known to result in hypoxia and release of potent angiogenic factors, even prolonged eye closure does not result in corneal neovascularization. This suggests that the closed eye tear film may contain factors that can impede neovascularization. Closed eye tear fluid is known to contain proteases capable of converting **plasminogen**/plasmin to angiostatin and other angiostatin-like A-chain fragments which are potent inhibitors of **angiogenesis**. This study was designed to characterize open and closed eye tear fluid for the presence of these entities. Methods. Open and closed eye tears were collected by microcapillaries from normal individuals. Tears were centrifuged and the supernatants analyzed by SDS-PAGE and western blotting. Membranes were probed with antibodies specific for the A-chain of plasmin and **plasminogen** and with antibodies specific for conformational domains on the smaller **N terminal** kringles 1.fwdarw.4 and kringles 1.fwdarw.3 fragments which are known **angiogenesis** inhibitors. Supernatants were also analyzed after fractionation by HPLC and binding to lysine sepharose 4B. The isolated fragments were identified based on size, lysine-binding capabilities, antigenic properties and by comparison with standards. Results. Open eye tear fluid from all normal individuals contained low levels of **plasminogen**, but no detectable antigens consistent with free A-chain or angiostatins. Tears collected after overnight eye closure contained significant amounts of **plasminogen**, A-chain antigen and various A-chain fragments including kringles 1.fwdarw.4 and kringles 1.fwdarw.3 and most likely free kringle 5, all known to have anti-**angiogenesis** properties. These were often present in concentrations likely to be physiologically significant. In samples collected from an atopic subject, the concentration of angiostatins in CTF increased markedly during active phases of the disease reaching levels of several ng/.mu.l. In these instances and in similar samples obtained from other atopic individuals experiencing active reactions, angiostatin was often detectable in basal-type tear fluid. Conclusion. A-chain fragments, which can inhibit **angiogenesis**, are often present at physiologically significant levels in human tear fluid collected after overnight eye closure. These fragments may play a role in preventing neovascularization in the hypoxic closed eye environment and may well be up regulated during inflammatory reactions.

L25 ANSWER 3 OF 3 MEDLINE DUPLICATE 1
1998030549 Document Number: 98030549. Angiostatin-converting enzyme activities of human matrilysin (MMP-7) and gelatinase B/type IV collagenase (MMP-9). Patterson B C; Sang Q A. (Department of Chemistry,

Florida State University, Tallahassee, Florida 32306-4390, USA.)JOURNAL OF BIOLOGICAL CHEMISTRY, (1997 Nov 14) 272 (46) 28823-5. Journal code: HIV. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Angiostatin is one of the most potent inhibitors of **angiogenesis** . Reports have shown that metalloelastase, pancreas elastase, plasmin reductase, and plasmin convert **plasminogen** to angiostatin. However, the cleavage sites of **plasminogen** by those enzymes have not been determined. Here we demonstrate that two members of the human matrix metalloproteinase (MMP) family, matrilysin (MMP-7) and gelatinase B/type IV collagenase (MMP-9), hydrolyze human **plasminogen** to generate angiostatin fragments. The cleavage sites have been determined. The 58-kDa bands derived from **plasminogen** by MMP-7 and MMP-9 both have the **N-terminal** sequence KVYLSEXKTG, which corresponds to that of angiostatin. This **N terminus** is identical to that of the starting **plasminogen** itself and corresponds to residues 97-106 of prepro-**plasminogen**. The 42- and 38-kDa bands generated by MMP-7 both have the **N-terminal** sequence VVLLPNVETP, which corresponds to the amino acid sequence 467-476 of prepro-**plasminogen**, between **kringle domain** 4 and 5. MMP-9 cleaves **plasminogen** to generate a 42-kDa fragment with the **N-terminal** sequence PVVLLPNVE, 1 residue upstream of the MMP-7 cleavage site. These results indicate that MMP-7 and MMP-9 may regulate new blood vessel formation by cleaving **plasminogen** and generating angiostatin molecules.

```
L26           6 FILE MEDLINE
L27          15 FILE CAPLUS
L28           5 FILE BIOSIS
L29           8 FILE EMBASE
L30           2 FILE WPIDS

TOTAL FOR ALL FILES
L31          36 L18 NOT L24

=> dup rem l31

PROCESSING COMPLETED FOR L31
L32          20 DUP REM L31 (16 DUPLICATES REMOVED)

=> d 1-20 cbib abs;s l12 and medicament
```

L32 ANSWER 1 OF 20 CAPLUS COPYRIGHT 2000 ACS

1999:571047 Document No. 131:295650 Role of endothelial cell extracellular signal-regulated kinase1/2 in urokinase-type **plasminogen** activator upregulation and in vitro **angiogenesis** by fibroblast growth factor-2. Giuliani, Roberta; Bastaki, Maria; Coltrini, Daniela; Presta, Marco (Unit of General Pathology and Immunology, Department of Biomedical, University of Brescia, Brescia, 25123, Italy). J. Cell Sci., 112(15), 2597-2606 (English) 1999. CODEN: JNCSAI. ISSN: 0021-9533. Publisher: Company of Biologists Ltd..

AB Downstream signaling triggered by the binding of fibroblast growth factor-2 (FGF2) to its tyrosine-kinase receptors involves the activation of mitogen-activated protein kinase kinase (MEK) with consequent phosphorylation of extracellular signal-regulated kinases (ERKs). FGF2 induces ERK1/2 activation in bovine aortic endothelial (BAE) cells and that the continuous presence of the growth factor is required for sustained ERK1/2 phosphorylation. This is prevented by the MEK inhibitors

PD 098059 and U0126, which also inhibit FGF2-mediated upregulation of urokinase-type **plasminogen** activator (uPA) and in vitro formation of capillary-like structures in three-dimensional type I collagen gel. Various FGF2 mutants originated by deletion or substitution of basic amino acid residues in the **N-terminus** or in the C-terminus of FGF2 retained the capacity to induce a long-lasting activation of ERK1/2 in BAE cells. Among them, K128Q/R129Q-FGF2 was also able to stimulate uPA prodn. and morphogenesis, whereas R129Q/K134Q-FGF2 caused uPA upregulation only. In contrast, K27,30Q/R31Q-FGF2, K128Q/K138Q-FGF2 and R118,129Q/K119,128Q-FGF2 exerted a significant uPA-inducing and morphogenic activity in an ERK1/2-dependent manner only in the presence of heparin. Furthermore, no uPA upregulation and morphogenesis was obsd. in BAE cells treated with the deletion mutant .DELTA.27-32-FGF2 even in the presence of sol. heparin. Thus, mutational anal. of FGF2 dissocs. the capacity of the growth factor to induce a persistent activation of ERK1/2 from its ability to stimulate uPA upregulation and/or in vitro **angiogenesis**. In conclusion, the data indicate that ERK1/2 phosphorylation is a key step in the signal transduction pathway switched on by FGF2 in endothelial cells. Nevertheless, a sustained ERK1/2 activation is not sufficient to trigger uPA upregulation and morphogenesis. FGF2 mutants may represent useful tools to dissect the signal transduction pathway(s) mediating the complex response elicited by an angiogenic stimulus in endothelial cells.

L32 ANSWER 2 OF 20 MEDLINE DUPLICATE 1

1999145535 Document Number: 99145535. Opposing actions of intact and **N-terminal** fragments of the human prolactin/growth hormone family members on **angiogenesis**: an efficient mechanism for the regulation of **angiogenesis**. Struman I; Bentzien F; Lee H; Mainfroid V; D'Angelo G; Goffin V; Weiner R I; Martial J A. (Laboratoire de Biologie Moleculaire et de Genie Genetique, Universite de Li`ege, Allee du 6 aout, B6, B-4000 Sart Tilman, Belgium.)PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1999 Feb 16) 96 (4) 1246-51. Journal code: PV3. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB **Angiogenesis**, the process of development of a new microvasculature, is regulated by a balance of positive and negative factors. We show both in vivo and in vitro that the members of the human prolactin/growth hormone family, i.e., human prolactin, human growth hormone, human placental lactogen, and human growth hormone variant are angiogenic whereas their respective 16-kDa **N-terminal** fragments are antiangiogenic. The opposite actions are regulated in part via activation or inhibition of mitogen-activated protein kinase signaling pathway. In addition, the **N-terminal** fragments stimulate expression of type 1 **plasminogen** activator inhibitor whereas the intact molecules have no effect, an observation consistent with the fragments acting via separate receptors. The concept that a single molecule encodes both angiogenic and antiangiogenic peptides represents an efficient model for regulating the balance of positive and negative factors controlling **angiogenesis**. This hypothesis has potential physiological importance for the control of the vascular connection between the fetal and maternal circulations in the placenta, where human prolactin, human placental lactogen, and human growth hormone variant are expressed.

L32 ANSWER 3 OF 20 MEDLINE

1999271801 Document Number: 99271801. Diurnal variations in angiostatin in human tear fluid: a possible role in prevention of corneal

neovascularization. Sack R A; Beaton A R; Sathe S. (SUNY College of Optometry, New York, NY 10010, USA.. RSack25968@AOL.com). CURRENT EYE RESEARCH, (1999 Mar) 18 (3) 186-93. Journal code: DUB. ISSN: 0271-3683. Pub. country: ENGLAND: United Kingdom. Language: English.

AB PURPOSE: Although overnight eye closure is known to result in hypoxia and release of potent angiogenic factors, even prolonged eye closure does not result in corneal neovascularization. This suggests that the closed eye tear film may contain factors that can impede neovascularization. Closed eye tear fluid is known to contain proteases capable of converting **plasminogen**/plasmin to angiostatin and other angiostatin-like A-chain fragments which are potent inhibitors of **angiogenesis**. This study was designed to characterize open and closed eye tear fluid for the presence of these entities. METHODS: Open and closed eye tears were collected by microcapillaries from normal individuals. Tears were centrifuged and the supernatants analyzed by SDS-PAGE and western blotting. Membranes were probed with antibodies specific for the A-chain of plasmin and **plasminogen** and with antibodies specific for conformational domains on the smaller **N terminal** kringles 1-->4 and kringles 1-->3 fragments which are known **angiogenesis** inhibitors. Supernatants were also analyzed after fractionation by HPLC and binding to lysine sepharose 4B. The isolated fragments were identified based on size, lysine-binding capabilities, antigenic properties and by comparison with standards. RESULTS: Open eye tear fluid from all normal individuals contained low levels of **plasminogen**, but no detectable antigens consistent with free A-chain or angiostatins. Tears collected after overnight eye closure contained significant amounts of **plasminogen**, A-chain antigen and various A-chain fragments including kringles 1-->4 and kringles 1-->3 and most likely free kringle 5, all known to have anti-**angiogenesis** properties. These were often present in concentrations likely to be physiologically significant. In samples collected from an atopic subject, the concentration of angiostatins in CTF increased markedly during active phases of the disease reaching levels of several ng/microl. In these instances and in similar samples obtained from other atopic individuals experiencing active reactions, angiostatin was often detectable in basal-type tear fluid. CONCLUSION: A-chain fragments, which can inhibit **angiogenesis**, are often present at physiologically significant levels in human tear fluid collected after overnight eye closure. These fragments may play a role in preventing neovascularization in the hypoxic closed eye environment and may well be up regulated during inflammatory reactions.

L32 ANSWER 4 OF 20 CAPLUS COPYRIGHT 2000 ACS

1999:323678 Document No. 131:180032 Studies on the antiangiogenic action of 16K PRL: regulation of urokinase-type **plasminogen** activator system. Lee, Hsinyu (Univ. of California, San Francisco, CA, USA). 153 pp. Avail. UMI, Order No. DA9903304 From: Diss. Abstr. Int., B 1999, 59(8), 3830 (English) 1998.

AB Unavailable

L32 ANSWER 5 OF 20 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 2

1998:239244 Document No. 128:281788 Methods and compositions for generating angiostatin. Soff, Gerald; Gately, Stephen T.; Twardowski, Przemyslaw (Northwestern University, USA; Soff, Gerald; Gately, Stephen T.; Twardowski, Przemyslaw). PCT Int. Appl. WO 9815574 A1 19980416, 98 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP,

KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1997-US16539 19970917. PRIORITY: US 1996-710305 19960917.

AB The invention provides methods of generating angiostatin in vivo comprising contacting **plasminogen** with a **plasminogen** activator and an -SH donor or contacting plasmin with an -SH donor. The invention also provides a method of treating angiogenic diseases by administering to an animal suffering from such a disease an -SH donor and, optionally, a **plasminogen** activator, **plasminogen**, or plasmin. The invention further comprises a compn. for generating angiostatin comprising an -SH donor and a **plasminogen** activator. The invention also provides a container holding an -SH donor and/or a **plasminogen** activator, said container having a label thereon instructing administration of the -SH donor and/or **plasminogen** activator to an animal suffering from an angiogenic disease. The invention further provides **plasminogen** fragments whose **N -terminal** amino acid is the same as that of plasmin and whose C-terminal amino acid is located in kringle 5 and which inhibit **angiogenesis**, antibodies which bind selectively to these fragments, methods and kits for using the antibodies, methods and materials for making the fragments by recombinant DNA techniques, and a method of treating an angiogenic disease comprising administering an effective amt. of one of the fragments. Finally, the invention provides a method of treating an angiogenic disease comprising administering a transgene coding for one of the fragments.

L32 ANSWER 6 OF 20 CAPLUS COPYRIGHT 2000 ACS

1998:728577 Document No. 130:485 Adenovirus-mediated intratumoral delivery of an **angiogenesis** antagonist for the treatment of tumors. Li, Hong; Lu, He; Griscelli, Franc; Opolon, Paule; Soria, Claudine; Ragot, Thierry; Legrand, Yves; Soria, Jeannette; Mabilat, Christelle; Perricaudet, Michel; Yeh, Patrice (Rhone-Poulenc Rorer S.A., Fr.). PCT Int. Appl. WO 9849321 A2 19981105, 59 pp. DESIGNATED STATES: W: AL, AU, BA, BB, BG, BR, CA, CN, CU, CZ, EE, GE, GW, HU, ID, IL, IS, JP, KR, LC, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, SL, TR, TT, UA, US, UZ, VN, YU, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1998-EP2491 19980427. PRIORITY: US 1997-44980 19970428.

AB A method for gene therapy of tumors that inhibits **angiogenesis** is described. A gene encoding an anti-angiogenic factor is introduced into tumor cells, for example with a defective adenovirus vector, to inhibit growth or metastasis, or both, of the tumor. Specifically, a defective adenovirus that carrying an expression cassette for the amino terminal fragment of urokinase (ATF) inhibited growth and metastasis of tumors. These effects were correlated with a remarkable inhibition of neovascularization within, and at the immediate vicinity of, the injection site. Delivery of a defective adenovirus vector that expresses kringles 1 to 3 of angiostatin inhibited tumor growth and tumorigenicity, and induced apoptosis of tumor cells. The invention further provides viral vectors for use in the methods of the invention.

L32 ANSWER 7 OF 20 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.

1998181812 EMBASE Angiostatin gene transfer: Inhibition of tumor growth in vivo by blockage of endothelial cell proliferation associated with a mitosis arrest. Griscelli F.; Li H.; Bennaceur-Griscelli A.; Soria J.; Opolon P.; Soria C.; Perricaudet M.; Yeh P.; Lu H.. F. Griscelli, Ctr. National Recherche Scientifique, Unite de Recherche Associee 1301, Rhone-Poulenc Rorer Gencell, 94805 Villejuif, France. grisceli@igr.fr. Proceedings of the National Academy of Sciences of the United States of America 95/11 (6367-6372) 26 May 1998.
Refs: 28.
ISSN: 0027-8424. CODEN: PNASA6. Pub. Country: United States. Language: English. Summary Language: English.

AB The antitumoral effects that follow the local delivery of the **N-terminal** fragment of human **plasminogen** (angiostatin K3) have been studied in two xenograft murine models. Angiostatin delivery was achieved by a defective adenovirus expressing a secretable angiostatin K3 molecule from the cytomegalovirus promoter (AdK3). In in vitro studies, AdK3 selectively inhibited endothelial cell proliferation and disrupted the G2/M transition induced by M-phase-promoting factors. AdK3-infected endothelial cells showed a marked mitosis arrest that correlated with the down-regulation of the M- phase phosphoproteins. A single intratumoral injection of AdK3 into preestablished rat C6 glioma or human MDA-MB-231 breast carcinoma grown in athymic mice was followed by a significant arrest of tumor growth, which was associated with a suppression of neovascularization within and at the vicinity of the tumors. AdK3 therapy also induced a 10-fold increase in apoptotic tumor cells as compared with a control adenovirus. Furthermore, we showed that systemic injection of AdK3 delayed C6 tumor establishment and growth, confirming that angiostatin can function in a paracrin manner. Our data support the concept that targeted antiangiogenesis, using adenovirus- mediated gene transfer, represents a promising alternative strategy for delivering antiangiogenic factors as their bolus injections present unsolved pharmacological problems.

L32 ANSWER 8 OF 20 MEDLINE DUPLICATE 3

1998389413 Document Number: 98389413. Inhibition of urokinase activity by the antiangiogenic factor 16K prolactin: activation of **plasminogen** activator inhibitor 1 expression. Lee H; Struman I; Clapp C; Martial J; Weiner R I. (Reproductive Endocrinology Center, University of California, San Francisco, 94143, USA.)ENDOCRINOLOGY, (1998 Sep) 139 (9) 3696-703.
Journal code: EGZ. ISSN: 0013-7227. Pub. country: United States.
Language:
English.

AB The **N-terminal** fragment of PRL (16K PRL) is an antiangiogenic factor that, in vitro, inhibits several components of **angiogenesis** including basic fibroblast growth factor (bFGF)-induced cell division, migration, and organization of capillary endothelial cells. An essential step in the regulation of **angiogenesis** is the activation of urokinase (urokinase type **plasminogen** activator, uPA), which in turn activates a cascade of proteases that play essential roles in endothelial cell migration and tissue remodeling. Treatment of bovine capillary endothelial cells (BBEC) with 16K PRL inhibited bFGF-stimulated urokinase activity in BBEC as detected by **plasminogen** substrate gel assay. 16K PRL did not appear to be acting via an effect on uPA expression because no change in messenger RNA levels were observed. However, protein levels of **plasminogen** activator inhibitor-1 (PAI-1), a specific inhibitor of urokinase, were increased by 16K PRL independent of the action of bFGF. The 16K PRL-induced increase in PAI-1 protein levels appear to be the result of increased expression of the PAI-1 gene. Increased production of PAI-1 induced by 16K PRL results in the formation of inactive PAI-1/uPA

complexes, consistent with the observed decrease in uPA activity.

L32 ANSWER 9 OF 20 CAPLUS COPYRIGHT 2000 ACS
1997:506600 Document No. 127:117379 Endothelial cell proliferation inhibitor
and method of use. Cao, Yihai; Folkman, Moses Judah (Children's Medical Center Corporation, USA). PCT Int. Appl. WO 9723500 A1 19970703, 51 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1996-US20447 19961213. PRIORITY: US 1995-8519 19951213; US 1996-763528 19961212.
AB The present invention comprises an endothelial inhibitor and method of use therefor. The endothelial cell proliferation inhibitor is a protein having a mol. wt. of approx. 14 kD and having an **N-terminal** sequence of GPVGAGEPKCPLMVKVLDAV, that has the ability to inhibit endothelial cell proliferation in in vitro assays.

L32 ANSWER 10 OF 20 CAPLUS COPYRIGHT 2000 ACS
1997:204264 Document No. 126:195235 Peptide analog inhibitors of urokinase receptor activity. Rosenberg, Steven; Spear, Kerry L.; Valerio, Robert; Bray, Andrew (Chiron Corporation, USA; Rosenberg, Steven; Spear, Kerry L.; Valerio, Robert; Bray, Andrew). PCT Int. Appl. WO 9705257 A1 19970213, 26 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1996-US12044 19960719. PRIORITY: US 1995-509208 19950731.
AB Peptides derived from the urokinase receptor and modified by the incorporation of D-amino acids or unusual L-amino acids are described for use as inhibitors of receptor function. These compds. are of potential use in the treatment of urokinase-modulated disorders, notably cancers, metastases, inflammation, **angiogenesis**, and inflammatory disease. The peptides are derived from the urokinase-binding peptide of the **N-terminal** growth factor-like domain. The peptides of the invention have IC50's for the receptor in the range 6.+-.1 - 240.+-.37 nM.

L32 ANSWER 11 OF 20 CAPLUS COPYRIGHT 2000 ACS
1997:748022 Document No. 128:97881 Stimulation of cell-surface urokinase-type **plasminogen** activator activity and cell migration in vascular endothelial cells by a novel hexapeptide analog of neurotensin. Ushiro, Shin; Mizoguchi, Kazushige; Yoshida, Shigeo; Jimi, Sei-ichiro; Fujiwara, Tadami; Yoshida, Masaya; Wei, Edward T.; Kitabgi, Patrick; Amagaya, Sakae; Ono, Mayumi; Kuwano, Michihiko (Maidashi, Department of Biochemistry, Kyushu University School of Medicine, Fukuoka 812-82, Japan). FEBS Lett., 418(3), 341-345 (English) 1997. CODEN: FEBLAL. ISSN: 0014-5793. Publisher: Elsevier Science B.V..
AB To investigate if neurotensin (NT) could induce activation of urokinase-type **plasminogen** activator (uPA) in vascular endothelial cells, the authors utilized the acetyl-NT (8-13) analog,

TJN-950, in which the C-terminal leucine is reduced to leucinol. TJN-950 inhibited the binding of 125I-NT to membranes of newborn rat brains and of COS-7 cells transfected with rat NT receptor cDNA, but at 104 higher doses than NT (8-13). However, TJN-950 was as effective as NT in inducing the fibrinolytic activity in bovine vascular aortic and human umbilical vein endothelial cells, and enhanced the migration of vascular endothelial cells. Moreover, administration of TJN-950 induced neovascularization in the rat cornea in vivo. TJN-950 had no effect on expression of uPA, **plasminogen** activator inhibitor-1 or uPA receptor mRNA. The binding of 125I-TJN-950 to cell membranes was blocked by unlabeled uPA and TJN-950, but not the **N-terminal** or 12-32 fragment of uPA. TJN-950 may enhance uPA activity in vascular endothelial cells by interacting with the uPA receptor, resulting in induction of **angiogenesis**.

L32 ANSWER 12 OF 20 CAPLUS COPYRIGHT 2000 ACS

1996:180603 Document No. 124:251678 Cooperative effect of TNF.alpha., bFGF, and VEGF on the formation of tubular structures of human microvascular endothelial cells in a fibrin matrix. Role of urokinase activity. Koolwijk, Pieter; van Erck, Monique G. M.; de Vree, Wil J. A.; Vermeer, Mario A.; Weich, Herbert A.; Hanemaaijer, Roeland; van Hinsbergh, Victor W. M. (Gaubius Laboratory, TNO-PG, Leiden, 2333 CK, Neth.). J. Cell Biol., 132(6), 1177-88 (English) 1996. CODEN: JCLBA3. ISSN: 0021-9525.

AB Three-dimensional fibrin matrixes were used to investigate the humoral requirements of human microvascular endothelial cells (hMVEC) to form capillary-like tubular structures. Basic FGF (bFGF) and VEGF165 were unable to induce tubular structures by themselves. Simultaneous addn. of one or both of these factors with tumor necrosis factor-.alpha. (TNF.alpha.) induced outgrowth of tubules, the effect being the strongest when bFGF, VEGF165, and TNF.alpha. were added simultaneously. Exogenously added urokinase-type **plasminogen** activator (u-PA), but not its nonproteolytic **N-terminal** fragment, could replace TNF.alpha., suggesting that TNF.alpha.-induced u-PA synthesis was involved. Sol. u-PA receptor (u-PAR) or antibodies that inhibited u-PA activity prevented the formation of tubular structures by 59-99%. .epsilon.-Aminocaproic acid and trasylol which inhibit the formation and activity of plasmin reduced the extent of tube formation by 71-95%. TNF.alpha. or u-PA did not induce tubular structures without addnl. growth factors. VEGF165 and bFGF enhanced of the u-PAR by 72 and 46%, but TNF.alpha. itself also increased u-PAR in hMVEC by 30%. Induction of mitogenesis was not the major contribution of bFGF and VEGF165 because the cell no. did not change significantly in the presence of TNF.alpha., and tyrphostin A47, which inhibited mitosis completely, reduced the formation of tubular structures only by 28-36%. These data show that induction of cell-bound u-PA activity by the cytokine TNF.alpha. is required in addn. to the angiogenic factors VEGF165 and/or bFGF to induce in vitro formation of capillary-like structures by hMVEC in fibrin matrixes. These data may provide insight in the mechanism of **angiogenesis** as occurs in pathol. conditions.

L32 ANSWER 13 OF 20 MEDLINE DUPLICATE 4

96195681 Document Number: 96195681. Limited plasmin proteolysis of vitronectin. Characterization of the adhesion protein as morpho-regulatory

and angiostatin-binding factor. Kost C; Benner K; Stockmann A; Linder D; Preissner K T. (Haemostasis Research Unit, Kerckhoff-Klinik, Bad Nauheim, Germany.)EUROPEAN JOURNAL OF BIOCHEMISTRY, (1996 Mar 1) 236 (2) 682-8. Journal code: EMZ. ISSN: 0014-2956. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB The adhesion protein vitronectin is associated with extracellular matrices and serves as cofactor for **plasminogen**-activator inhibitor-1. Limited proteolysis by plasmin converts vitronectin into defined fragments which are detectable at sites of inflammation and **angiogenesis**. The loss and gain of binding functions of vitronectin fragments for macromolecular ligands was characterized in the present study. The initially generated 61--63-kDa vitronectin-(1--348)-fragment serves as typical binding component for **plasminogen** and binding function was lost upon carboxypeptidase B treatment indicating the importance of a C-terminal lysine. Complementary binding sites reside in isolated **plasminogen** kringles 1--3 (designated angiostatin) as deduced from direct binding and ligand blotting experiments. A synthetic vitronectin-(331--348)-peptide from the C-terminus of the 61--63-kDa fragment could mimic **plasminogen** and angiostatin binding. Also, the immobilized peptide bound tissue **plasminogen**-activator and mediated plasmin formation, comparable to fibrinogen-derived peptides. The 61--63-kDa vitronectin fragment was indistinguishable in its adhesive properties to intact vitronectin and bound active but not latent **plasminogen**-activator inhibitor-1. Late plasminolysis of vitronectin resulted in the processing of the **N-terminal** region of the protein with the generation of 42 kDa/35-kDa fragments that had Gly89 as new **N-terminus** and that were ineffective in promoting cell adhesion. Thus, at sites of cell-matrix interactions which become proteolytically modified by plasmin during inflammatory and angiogenic processes, vitronectin serves as **plasminogen** /angiostatin-binding factor. Due to this differential change in functions particularly at sites of deposition in the vascular system or at wound sites vitronectin is considered to be an important morpho-regulatory factor.

L32 ANSWER 14 OF 20 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.

96042558 EMBASE Document No.: 1996042558. Inductive influences of epimorphin on endothelial cells in vitro. Oka Y.; Hirai Y.. Biomedical Research and Development, Sumitomo Electric Industries, 1 Taya-cho, Sakae-ku,Yokohama 244, Japan. Experimental Cell Research 222/1 (189-198) 1996. ISSN: 0014-4827. CODEN: ECREAL. Pub. Country: United States. Language: English. Summary Language: English.

AB Epimorphin is known as a mesenchymal factor involved in epithelial morphogenesis. This protein has, however, a curious nature in that only certain of its molecules are transported to extracellular regions after having undergone complex conformational changes. In the present study, we generated an **N-terminally** modified recombinant epimorphin fragment as a substitute for the extracellular epimorphin and examined in detail how this polypeptide affects cellular behavior in a model cell system. As immunohistochemical studies revealed that epimorphin is abundant in regions close to endothelial cells in venulae, we chose endothelial cells as the model cell and investigated the influence of this polypeptide on their cellular behavior in vitro. The recombinant epimorphin guided the endothelial cells to align themselves in tandem and to present a branched morphology in the three-dimensional culture system. We also discovered that the endothelial cells were induced to secrete

several cytokines, including those involved in **angiogenesis**, and were suppressed in terms of proliferation by this molecule. These results suggest that epimorphin has a regulatory role in the activation of endothelial cells and is active in supporting the resulting cellular arrangement.

L32 ANSWER 15 OF 20 CAPLUS COPYRIGHT 2000 ACS
1996:142629 Document No. 124:256682 Blockage of urokinase receptor reduces in vitro the motility and the deformability of endothelial cells. Lu, He;
Mabilat, Christelle; Yeh, Patrice; Guitton, Jean-Dominique; Li, Hong; Pouchelet, Marcel; Shoevaert, Damien; Legrand, Yves; Soria, Jeannette; et al. (INSERM U353, Hopital St. Louis, 1 Avenue Claude Vellefaux, Paris, 75010, Fr.). FEBS Lett., 380(1,2), 21-4 (English) 1996. CODEN: FEBLAL. ISSN: 0014-5793.
AB Binding of urokinase (u-PA) to its cell surface receptor (u-PAR) is crit. for tumor cell invasion. Disruption of this binding by an u-PAR antagonist ATF-HSA (**N-terminal** residues 1-135 of u-PA conjugated to human serum albumin) inhibits in vitro the motility of endothelial cells in a dose-dependent manner. This inhibition was also obsd. when cells were first stimulated with potent angiogenic factors, including basic fibroblast growth factor or vascular endothelial growth factor. [3H]thymidine incorporation assay demonstrated that ATF-HSA did not affect cell proliferation. ATF-HSA was more potent than plasmin inhibitors, suggesting that it exerts its effects not solely by inhibiting
the remodeling of the extracellular matrix. In fact, anal. of cell shape change during migration revealed that its effect is related to a decrease in cell deformability. These results suggest that u-PAR antagonist may be
a new approach to control **angiogenesis**.

L32 ANSWER 16 OF 20 CAPLUS COPYRIGHT 2000 ACS
1996:28733 Document No. 124:113640 Regulation of mesothelial cell mitogenesis by antisense oligonucleotides for the urokinase receptor. Shetty, Sreerama; Kumar, Anuradha; Johnson, Alice R.; Idell, Steven (University of Texas Health Center at Tyler, Tyler, TX, 75710, USA). Antisense Res. Dev., 5(4), 307-14 (English) 1995. CODEN: AREDEI. ISSN: 1050-5261.
AB The assocn. of urokinase-type **plasminogen** activator (uPA) with its receptor (uPAR) influences various biol. functions, including cell migration, **angiogenesis**, differentiation, and wound healing. Expression of uPAR at the mesothelial surface could, therefore, influence cellular responses in the pleural space. The authors found that a line of
cultured human mesothelial cells (MeT5A) expressed specific and saturable binding sites for .mu.PA that increased on stimulation with PMA. Ligand blotting studies showed that the mesothelial receptor is a 50 kDa protein similar to that in other cell lines. Binding of active and intact, but not **N-terminal** or low mol. wt. fragment, .mu.PA to mesothelial cells enhanced DNA synthesis and cell proliferation, and antibodies against either the active site of uPA or uPAR abrogated this effect. The authors reasoned that regulation of uPAR expression could control uPA-induced mitogenesis and tested this hypothesis with antisense oligonucleotides complementary to uPAR mRNA. Phosphorothioate-modified antisense oligonucleotides inhibited uPA-mediated mesothelial cell proliferation in a concn.-dependent manner. These effects were assocd. with decreased binding of 125I-uPA and reduced expression of the uPAR gene
product. The results indicate that uPAR is involved in signal transduction pathways that control uPA-mediated mesothelial cell

proliferation, a process implicated in the pathogenesis of mesothelial inflammation and pleural neoplasia. Antisense oligonucleotides to uPAR suppress mesothelial cell mitogenesis in vitro and offer a potential means of regulating the process in vivo.

L32 ANSWER 17 OF 20 MEDLINE DUPLICATE 5
95080428 Document Number: 95080428. Blockage of the urokinase receptor on the cell surface: construction and characterization of a hybrid protein consisting of the **N-terminal** fragment of human urokinase and human albumin. Lu H; Yeh P; Guitton J D; Mabilat C; Desanlis F; Maury I; Legrand Y; Soria J; Soria C. (Unite INSERM 353, Hopital St. Louis, Paris, France.)FEBS LETTERS, (1994 Dec 12) 356 (1) 56-9. Journal code: EUH. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.
AB Receptor-bound urokinase is likely to be a crucial determinant in both tumor invasion and **angiogenesis**. We report here that a yeast-derived genetic conjugate between human serum albumin and the 1-135 **N-terminal** residues of urokinase (u-PA) competitively inhibits the binding of exogenous and endogenous u-PA to its cell-anchored receptor (u-PAR). This hybrid molecule (ATF-HSA) also inhibits in vitro pro-urokinase-dependent **plasminogen** activation in the presence of u-PAR bearing cells. These effects are probably responsible for the observed in vitro inhibition of tumor cell invasion in a reconstituted basement membrane extract (Matrigel).

L32 ANSWER 18 OF 20 MEDLINE DUPLICATE 6
92355653 Document Number: 92355653. Regulation of gene expression by SPARC during **angiogenesis** in vitro. Changes in fibronectin, thrombospondin-1, and **plasminogen** activator inhibitor-1. Lane T F; Iruela-Arispe M L; Sage E H. (Department of Biological Structure, University of Washington, Seattle 98195.)JOURNAL OF BIOLOGICAL CHEMISTRY, (1992 Aug 15) 267 (23) 16736-45. Journal code: HIV. ISSN: 0021-9258. Pub. country: United States. Language: English.
AB **Angiogenesis** in vitro, the formation of capillary-like structures by cultured endothelial cells, is associated with changes in the expression of several extracellular matrix proteins. The expression of SPARC, a secreted collagen-binding glycoprotein, has been shown to increase significantly during this process. We now show that addition of purified SPARC protein, or an **N-terminal** synthetic peptide (SPARC4-23), to strains of bovine aortic endothelial cells undergoing **angiogenesis** in vitro resulted in a dose-dependent decrease in the synthesis of fibronectin and thrombospondin-1 and an increase in the synthesis of type 1-**plasminogen** activator inhibitor. SPARC decreased fibronectin mRNA by 75% over 48 h, an effect that was inhibited by anti-SPARC immunoglobulins. Levels of thrombospondin-1 mRNA were diminished by 80%. Over a similar time course, both mRNA and protein levels of type 1-**plasminogen** activator inhibitor (PAI-1) were enhanced by SPARC and the SPARC4-23 peptide. The effects were dose-dependent with concentrations of SPARC between 1 and 30 micrograms/ml. In contrast, no changes were observed in the levels of either type I collagen mRNA or secreted gelatinases. Half-maximal induction of PAI-1 mRNA or inhibition of fibronectin and thrombospondin mRNAs occurred with 2-5 micrograms/ml SPARC and approximately 0.05 mM SPARC4-23. Strains of endothelial cells that did not form cords and tubes in vitro had reduced or undetectable responses to SPARC under identical conditions. These results demonstrate that SPARC modulates the synthesis

of a subset of secreted proteins and identify an **N-terminal** acidic sequence as a region of the protein that provides an active site. SPARC might therefore function, in part, to achieve an optimal ratio among different components of the extracellular matrix. This activity would be consistent with known effects of SPARC on cellular morphology and proliferation that might contribute to the regulation of **angiogenesis** in vivo.

L32 ANSWER 19 OF 20 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
92063178 EMBASE Document No.: 1992063178. Characterization and regulation of the urokinase receptor of human endothelial cells. Barnathan E.S.. 919 Gates Pavilion, Hospital of the University of Pennsylvania, 3400 Spruce Street, Philadelphia, PA 19104, United States. Fibrinolysis 6/SUPPL. 1 (1-9) 1992.
ISSN: 0268-9499. CODEN: FBRIE7. Pub. Country: United Kingdom. Language: English. Summary Language: English.
AB Endothelial cells synthesize and secrete urokinase-type **plasminogen** activators (u-PA) in response to various stimuli. To modulate the local expression of u-PA activity both intravascularly and pericellularly during **angiogenesis**, endothelial cells express both inhibitors (primarily PAI-1) and receptors (u-PAR) for u-PA. The interaction of u-PA with receptors on the surface of endothelial cells may play an important role in the regulation of fibrinolysis and cell migration. Using radioligand binding studies, we and others have demonstrated that human umbilical vein endothelial cells (HUVEC) express high affinity receptors for urokinase on the cell surface. We have demonstrated that single chain urokinase (scu-PA, prourokinase) binds only via the growth factor domain, while two chain high molecular weight urokinase (tcu-PA) can bind to the receptor or to cell- or matrix-associated PAI-1. We have isolated a .apprx.46kDa glycoprotein from HUVEC using affinity chromatography which retains the ability to specifically bind u-PA. At least three post-translational modifications appear to occur including removal of an **N-terminal** signal peptide; N-linked glycosylation, and C-terminal cleavage with addition of a phosphatidylinositol-glycan moiety which links the externally oriented protein to the cell surface. Using the polymerase chain reaction and published sequence information of the u-PAR cloned from a transformed fibroblast cDNA library, we amplified cDNAs of u-PAR from HUVEC and PMA-treated U937 cells. The specificity of the cDNAs was confirmed by restriction mapping and direct sequence analysis. Using these probes and radioligand binding studies we have demonstrated that at least two independent protein kinase pathways exist in endothelial cells for upregulating u-PA receptor expression. Down regulation of receptors may be pathophysiologic in thrombotic disorders whereas up regulation may be important in promoting wound healing, vascular repair, and protection from thrombosis. Up regulation could be harmful as well in such conditions as pathologic neovascularization (e.g., diabetic retinopathy) and in tumour metastasis as well as in tumour-related **angiogenesis**.
Understanding the control and functional significance of u-PA binding to cells in general will hopefully enable the design of therapies to optimize the beneficial aspects and minimize any harmful effects of this interaction. Changes in the local expression of u-PA, PAI-1 and u-PAR by

endothelial cells may affect the extent of **angiogenesis** or the degree of local intravascular fibrinolysis, which might be critical in conditions such as unstable angina and myocardial infarction.

L32 ANSWER 20 OF 20 WPIDS COPYRIGHT 2000 DERWENT INFORMATION LTD
AN 1989-167092 [23] WPIDS
AB EP 319052 A UPAB: 19930923
Recombinant human mutant, biologically active, microheterogeneous forms of
acidic fibroblast growth factor (aFGF) are claimed. The aFGF may have a substn of cysteine residue(s) at positions 16, 83 and 117, numbered in accordance with the native human 140 amino acid microheterogeneous form, with an amino acid incapable of forming intramolecular or intermolecular disulphide bonds and opt having an additional methionine attached to the ordinarily first amino acid at the **N-terminus**.

Also claimed is recombinant bovine mutant, biologically active, microheterogeneous aFGF comprising the substitution of cysteine residue(s), at positions 16, 47 and 83, numbered in accordance with the native bovine 140 amino acid microheterogeneous form, with an amino acid incapable of forming intramolecular or intermolecular disulphide bonds and
opt having an additional methionine attached to the ordinarily first amino
acid at the **N-terminus**.

USE/ADVANTAGE - The mutated forms of aFGF have increased biological activity in the presence or absence of heparin compared to native aFGF. They may be used for promoting the repair or healing of soft tissue wounds
resulting from burns, cuts or lacerations and cutaneous ulcerations along with musculo-skeletal wounds such as bone fractures, ligament and tendon tears and inflammation of bursas and tendons. They may also be used to promote in vivo vascular tissue repair, promoting central and peripheral nerve tissue repair and for the in vivo induction of **plasminogen** activator by vascular endothelial cells for the treatment of thrombotic attacks. They may also be used for in vitro growth of endothelial cells.
0/0
ABEQ US 5223483 A UPAB: 19931116
Recombinant human mutant, biologically active, microheterogeneous forms of
aFGF comprise Cys-16, -83 and -117 replaced with an amino acid incapable of forming intramolecular or intermolecular S-S bonds. Mutant is less dependent on heparin and has an optional **N-terminal** methionine residue.

USE/ADVANTAGE - Mutant has increased biological activity, and is used
for promoting tissue repair.
Dwg.0/2
ABEQ US 5312911 A UPAB: 19940627
Nucleotide sequence codes for human mutant microheterogeneous forms of acidic fibroblast growth factor, such that all 3 Cys residues at positions
16, 83 and 117 (of the 140 residue polypeptide) are replaced with a residue which cannot form intra- or inter-molecular S-S bonds.

Opt. prod. has an additional Met residue attached to the **N-terminus**.

ADVANTAGE - Prod. has increased biological activity and less dependence on heparin w.r.t. native growth factor.
Dwg.0/2
ABEQ EP 319052 B UPAB: 19950301
A recombinant human, biologically active, form of acidic fibroblast growth

factor protein, 154, 140 or 139 amino acids in length, produced from a single gene unit of DNA, said protein comprising the substitution of one or more of the cysteine residues at positions 16, 83 and 117 of the corresponding native protein, numbered in accordance with the native human form of acidic fibroblast growth factor 140 amino acis in length which has the amino acid sequence dfined in the specification comprising 140 aminoacids with an amino acid incapable of forming intramolecular or intermolecular disulfide bonds.
Dwg.0/2

ABEQ US 5409897 A UPAB: 19950609
Promotion of nutogenesis comprises admin. of a recombinant human microheterogeneous form of aFGF. All 3 Cys residues at positions 16, 83 and 117 are replaced with an aminoacid incapable of forming intramolecular or intermolecular S-S bonds. A FGF may have an additional Met on the **N-terminus** of the microheterogeneous form.
USE - Repairing tissues, e.g. skin wounds, bone fractures, ligaments and tendon tears, inflammation, repairing corneal tissue, promoting **angiogenesis**, treating thrombotic attacks, digesting preformed clots and prevention of further attacks, repairing neurons, e.g. damaged or destroyed in Alzheimer's disease, etc.
Dwg.0/2

```
L33          0 FILE MEDLINE
L34          2 FILE CAPLUS
L35          0 FILE BIOSIS
L36          0 FILE EMBASE
L37          2 FILE WPIDS

TOTAL FOR ALL FILES
L38          4 L12 AND MEDICAMENT

=> dup rem l38

PROCESSING COMPLETED FOR L38
L39          4 DUP REM L38 (0 DUPLICATES REMOVED)

=> d 1-4 cbib abs
```

L39 ANSWER 1 OF 4 CAPLUS COPYRIGHT 2000 ACS
1999:811356 Document No. 132:46609 sequence and therapeutic applications for human angiostatin-binding protein. Holmgren, Lars; Troyanovsky, Boris (Pharmacia & Upjohn AB, Swed.). PCT Int. Appl. WO 9966038 A1 19991223, 58 pp. DESIGNATED STATES: W: AL, AU, BA, BB, BG, BR, CA, CN, CU, CZ, EE, GD, GE, HR, HU, ID, IL, IN, IS, JP, KE, KP, KR, LC, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, SL, TR, TT, UA, US, UZ, VN, YU, ZA, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-EP4109 19990611. PRIORITY: SE 1998-2130 19980615; US 1998-89266 19980615; SE 1998-4372 19981217; US 1998-114386 19981229.

AB The present invention provides the sequence of a protein capable of acting as an angiostatin receptor as well as the nucleic acid sequence thereof.

This protein is named ABP-1 defined by its ability to bind a fragment of **plasminogen** preferably by the first four Kringle domains. Binding and signaling of angiostatin was shown via the ABP-1 protein. ABP-1 mediates angiostatin-induced focal adhesion kinase activity. The sequence of Big 3 (angiostatin-binding domain) is also provided. The invention also relates to the use thereof in screening methods, wherein novel substances are created exhibiting the same advantageous anti-angiogenic properties as angiostatin. Evidence that angiostatin binds to endothelial cells is provided.

L39 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2000 ACS
1999:42589 Document No. 130:90516 Use of bromelain in the manufacture of a **medicament** for enhancement of intestinal permeability. Mynott, Tracey Lehanne; Fasano, Alessio (Cortecs (UK) Limited, UK; The University of Maryland at Baltimore). PCT Int. Appl. WO 9900141 A1 19990107, 31 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1998-GB1895 19980626. PRIORITY: GB 1997-13667 19970627.
AB Bromelain (I) is capable of enhancing the permeability of the intestine and therefore is able to increase the absorption of proteins such as insulin and other macromol. biol. active agents. Rabbits' intestinal epithelium treatment with 15 mg/mL I increased intestinal permeability in a dose-dependent manner, which was reversed when I was removed. I did not have an adverse effect on nutrient influx suggesting that the use of this substance was safe.

L39 ANSWER 3 OF 4 WPIDS COPYRIGHT 2000 DERWENT INFORMATION LTD
AN 1995-067160 [09] WPIDS
AB WO 9502413 A UPAB: 19950306
Inhibition of malignant tumour growth, invasion and/or metastasis in a patient who has or has a high risk of developing a tumour comprises suppressing the inhibitory action of an inhibitor of a protease (PI) or of a non-proteolytic matrix degrading enzyme (MDE) in (potentially) malignant tissue. Also claimed are: (1) methods for identifying cpds. comprising: (i) adding the cpd. to a system comprising PI/MDE and protease/MDE (one of these being immobilised, the other labelled) where the protease/MDE bound to PI/MDE is detected by a labelled enzyme or by a labelled antibody (Ab) directed against PI/MDE; (ii) adding the cpd. to a system comprising radiolabelled inhibitor of PI/MDE and tumour cells expressing the protease/MDE and measuring inhibition of binding by gamma counting of the cells; (iii) using whole cells having a surface receptor for the protease/MDE (based on inhibition of receptor-bound uPA; inhibition of plasmin generation is measured with the plasmin-specific fluorogenic substrate H-D-Val-Leu-Lys-7-amido- 4-methyl-coumarin); (2) admin. of the cpd. to nude mice or rats inoculated with human malignant tumour cells capable of invasion and/or metastasis in the presence of the protease or MDE and PI or MDE, and selecting those that inhibit the growth of these cells; (3) a cpd. which is a suppressor of an inhibitor of a protease/MDE in malignant tumour tissue for use as a **medicament**; (4) the use

of these cpds. for preparing a compsn. for inhibiting malignant tumour growth, invasion and/or metastasis in a patient who has been established to have a high risk of developing a malignant tumour or who has developed a malignant tumour.

USE - Suppression of PI allows the protease/MDE to degrade tumour tissue or to interfere with tumour **angiogenesis** and/or migration of tumour cells. The method can be a neoadjuvant, preoperative and/or adjuvant treatment in patients (a) with established cancer (e.g. carcinoma of the breast, prostate or bladder; non-small cell lung tumour, colonic adenocarcinoma; lymphoma, melanoma etc.) or (b) assessed as being as risk because of high levels of tumour-associated markers or gene product (esp. high levels of PI). No dosage was given. (I) are usually administered by injection.

ADVANTAGE - The method only inhibits malignant cells and has no significant effect on stromal cells.

Dwg.0/6

L39 ANSWER 4 OF 4 WPIDS COPYRIGHT 2000 DERWENT INFORMATION LTD
AN 1988-243636 [35] WPIDS
AB EP 280135 A UPAB: 19930923

The use of protein C-inhibitor (I) as a pharmaceutical is new. Also new is labelled (I) itself. (I) forms inactive complexes with **plasminogen** activators, which are involved in the growth and metastatis of tumours. In the presence of a sulphated saccharide (A), the reaction between (I) and activators is greatly accelerated, and preferential reaction with urokinase occurs.

USE - (I) is useful for treatment and invitro and invivo diagnosis of angiogenic diseases (e.g. diabetic retinopathy, neovascular glaucoma, rheumatic arthritis or psoriasis) and particularly tumours.

0/0

ABEQ EP 280135 B UPAB: 19930923

Protein C inhibitor for use as **medicament**.

=> s holmgren l?/au,in;s troyanovsky b?/au,in

'IN' IS NOT A VALID FIELD CODE
L40 33 FILE MEDLINE
L41 51 FILE CAPLUS
L42 36 FILE BIOSIS
'IN' IS NOT A VALID FIELD CODE
L43 25 FILE EMBASE
L44 7 FILE WPIDS

TOTAL FOR ALL FILES
L45 152 HOLMGREN L?/AU,IN

'IN' IS NOT A VALID FIELD CODE
L46 0 FILE MEDLINE
L47 3 FILE CAPLUS
L48 0 FILE BIOSIS
'IN' IS NOT A VALID FIELD CODE
L49 0 FILE EMBASE
L50 4 FILE WPIDS

TOTAL FOR ALL FILES

```
L51             7 TROYANOVSKY B?/AU,IN

=> s l45 and l51

L52             0 FILE MEDLINE
L53             1 FILE CAPLUS
L54             0 FILE BIOSIS
L55             0 FILE EMBASE
L56             0 FILE WPIDS

TOTAL FOR ALL FILES
L57             1 L45 AND L51

=> d cbib abs


L57  ANSWER 1 OF 1  CAPLUS  COPYRIGHT 2000 ACS
1999:811356   Document No. 132:46609  sequence and therapeutic applications
for
     human angiostatin-binding protein.  Holmgren, Lars; Troyanovsky,
     Boris (Pharmacia & Upjohn AB, Swed.).  PCT Int. Appl. WO 9966038 A1
     19991223, 58 pp.  DESIGNATED STATES: W:  AL, AU, BA, BB, BG, BR, CA, CN,
     CU, CZ, EE, GD, GE, HR, HU, ID, IL, IN, IS, JP, KE, KP, KR, LC, LK, LR,
     LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, SL, TR, TT, UA, US,
     UZ, VN, YU, ZA, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ,
     CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC,
     ML, MR, NE, NL, PT, SE, SN, TD, TG.  (English).  CODEN: PIXXD2.
     APPLICATION: WO 1999-EP4109 19990611.  PRIORITY: SE 1998-2130 19980615;
US
     1998-89266 19980615; SE 1998-4372 19981217; US 1998-114386 19981229.
AB   The present invention provides the sequence of a protein capable of
acting
     as an angiostatin receptor as well as the nucleic acid sequence thereof.
     This protein is named ABP-1 defined by its ability to bind a fragment of
     plasminogen preferably by the first four Kringle domains.  Binding and
     signaling of angiostatin was shown via the ABP-1 protein.  ABP-1 mediates
     angiostatin-induced focal adhesion kinase activity.  The sequence of Big
3
     (angiostatin-binding domain) is also provided.  The invention also
relates
     to the use thereof in screening methods, wherein novel substances are
     created exhibiting the same advantageous anti-angiogenic properties as
     angiostatin.  Evidence that angiostatin binds to endothelial cells is
     provided.


=> s angiogenes? and (145 or 151)

L58             8 FILE MEDLINE
L59            11 FILE CAPLUS
L60            11 FILE BIOSIS
L61             9 FILE EMBASE
L62             0 FILE WPIDS

TOTAL FOR ALL FILES
L63            39 ANGIOGENES? AND (L45 OR L51)

=> s l63 not (138 or 157)

L64             8 FILE MEDLINE
L65            10 FILE CAPLUS
```

L66 11 FILE BIOSIS
L67 9 FILE EMBASE
L68 0 FILE WPIDS

TOTAL FOR ALL FILES
L69 38 L63 NOT (L38 OR L57)

=> dup rem l69

PROCESSING COMPLETED FOR L69
L70 15 DUP REM L69 (23 DUPLICATES REMOVED)

=> d 1-16 cbib abs;del his y;fil reg

L70 ANSWER 1 OF 15 MEDLINE DUPLICATE 1
1999061790 Document Number: 99061790. Multiple forms of angiostatin induce apoptosis in endothelial cells. Lucas R; **Holmgren L**; Garcia I; Jimenez B; Mandriota S J; Borlat F; Sim B K; Wu Z; Grau G E; Shing Y; Soff G A; Bouck N; Pepper M S. (Laboratory of Immunopathology of Intensive Care, Department of Anesthesiology, Geneva University Hospital, Geneva, Switzerland.)BLOOD, (1998 Dec 15) 92 (12) 4730-41. Journal code: A8G. ISSN: 0006-4971. Pub. country: United States. Language: English.
AB Angiostatin is a circulating inhibitor of **angiogenesis** generated by proteolytic cleavage of plasminogen. In this study we have used recombinant human and murine angiostatins (kringles 1-4) as well as native human angiostatin (prepared by elastase digestion of plasminogen [kringles 1-3] or by plasmin autocatalysis in the presence of a free sulfhydryl donor [kringles 1-4]). We report that angiostatin reduces endothelial cell number in a 4-day proliferation assay without affecting cell cycle progression into S-phase (as determined by bromodeoxyuridine labeling). This suggested that the reduction in cell number in the proliferation assay might in part be due to cytotoxicity. This was confirmed by the observation that ethidium homodimer incorporation (a measure of plasma membrane integrity) into endothelial cells was increased by angiostatin in a manner similar to that seen with tumor necrosis factor- (TNF-) and transforming growth factor-beta1 (TGF-beta1), both of which induce apoptosis in endothelial cells. In contrast to TNF- and TGF-beta1, angiostatin did not induce cytotoxicity in human MRC-5 fibroblast, rat smooth muscle, canine MDCK epithelial, or murine B16-F10 melanoma cell lines. Angiostatin-induced apoptosis was confirmed by endothelial cell nuclear acridine orange incorporation as well as by annexin V and TUNEL staining. These in vitro findings point to endothelial cell apoptosis as a mechanism for the antiangiogenic effect of angiostatin in vivo.

L70 ANSWER 2 OF 15 MEDLINE DUPLICATE 2
1998451278 Document Number: 98451278. p53 induces **angiogenesis**-restricted dormancy in a mouse fibrosarcoma. **Holmgren L**; Jackson G; Arbiser J. (Microbiology and Tumor Biology Center, Karolinska Institute, Stockholm, Sweden.)ONCOGENE, (1998 Aug 20) 17 (7) 819-24. Journal code: ONC. ISSN: 0950-9232. Pub. country: ENGLAND: United Kingdom.
Language: English.
AB The p53 tumor-suppressor gene is inactivated in over 50% of all human cancers. In normal cells, p53 induces growth arrest and apoptosis in

response to DNA damage. We show that p53 acts as potent tumor-suppressor gene independent of its well-documented effects on tumor-cell proliferation and apoptosis. p53 activates target genes in a murine fibrosarcoma cell-line but does not affect tumor cell-cycle progression or survival. Exogenous expression of wt-p53 does, however, block the angiogenic potential of the tumor cells resulting in formation of dormant tumors in vivo. These data provide evidence that: (1) p53 acts as a tumor suppressor gene independent of its anti-proliferative effects; (2) By inhibiting **angiogenesis** p53 can indirectly induce apoptosis in vivo but not in vitro; (3) p53-gene therapy which alters a tumors angiogenic potential, can revert tumors to a dormant phenotype.

L70 ANSWER 3 OF 15 CAPLUS COPYRIGHT 2000 ACS
1998:238978 Document No. 128:316810 Inhibition of tumor **angiogenesis** and the induction of tumor dormancy. **Holmgren, Lars**; Bicknell, Roy (Department of Tumour Biology, Karolinska Institute, Stockholm, Swed.). Tumour Angiog., 301-307. Editor(s): Bicknell, Roy; Lewis, Claire E.; Ferrara, Napoleone. Oxford University Press: Oxford, UK. (English) 1997. CODEN: 65VVA7.
AB A review with 51 refs.

L70 ANSWER 4 OF 15 MEDLINE DUPLICATE 3
96312529 Document Number: 96312529. Antiangiogenic therapy of transgenic mice impairs de novo tumor growth. Parangi S; O'Reilly M; Christofori G; **Holmgren L**; Grosfeld J; Folkman J; Hanahan D. (Department of Biochemistry and Biophysics, Hormone Research Institute, University of California, San Francisco, CA 94143-0534, USA.)PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1996 Mar 5) 93 (5) 2002-7. Journal code: PV3. ISSN: 0027-8424. Pub. country: United States. Language: English.
AB **Angiogenesis** is activated during multistage tumorigenesis prior to the emergence of solid tumors. Using a transgenic mouse model, we have tested the proposition that treatment with **angiogenesis** inhibitors can inhibit the progression of tumorigenesis after the switch to the angiogenic phenotype. In this model, islet cell carcinomas develop from multifocal, hyperproliferative nodules that show the histological hallmarks of human carcinoma in situ. Mice were treated with a combination of the **angiogenesis** inhibitor AGM-1470 (TNP-470), the antibiotic minocycline, and interferon alpha/beta. The treatment regimen markedly attenuated tumor growth but did not prevent tumor formation; tumor volume was reduced to 11% and capillary density to 40% of controls. The proliferation index of tumor cells in treated and control mice was similar, whereas the apoptotic index was doubled in treated tumors. This study shows that de novo tumor progression can be restricted solely by antiangiogenic therapy. The results suggest that **angiogenesis** inhibitors represent a valid component of anticancer strategies aimed at progression from discrete stages of tumorigenesis and demonstrate that transgenic mouse models can be used to evaluate efficacy of candidate antiangiogenic agents.

L70 ANSWER 5 OF 15 MEDLINE DUPLICATE 4
96233652 Document Number: 96233652. Angiostatin induces and sustains dormancy of human primary tumors in mice. O'Reilly M S; **Holmgren L**; Chen C; Folkman J. (Department of Surgery, Children's Hospital, Boston, Massachusetts, USA.)NATURE MEDICINE, (1996 Jun) 2 (6) 689-92. Journal code: CG5. ISSN: 1078-8956. Pub. country: United States. Language: English.

AB There is now considerable direct evidence that tumor growth is **angiogenesis**-dependent. The most compelling evidence is based on the discovery of angiostatin, an **angiogenesis** inhibitor that selectively instructs endothelium to become refractory to angiogenic stimuli. Angiostatin, which specifically inhibits endothelial proliferation, induced dormancy of metastases defined by a balance of apoptosis and proliferation. We now show that systemic administration of human angiostatin potently inhibits the growth of three human and three murine primary carcinomas in mice. An almost complete inhibition of tumor growth was observed without detectable toxicity or resistance. The human carcinomas regressed to microscopic dormant foci in which tumor cell proliferation was balanced by apoptosis in the presence of blocked **angiogenesis**. This regression of primary tumors without toxicity has not been previously described. This is also the first demonstration of dormancy therapy, a novel anticancer strategy in which malignant tumors are regressed by prolonged blockade of **angiogenesis**.

L70 ANSWER 6 OF 15 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 5
1996:517601 Document No. 125:164512 Anti-**angiogenesis** restricted tumor dormancy. **Holmgren, Lars** (Microbiology and Tumor Biology Center, Karolinska Institute, Stockholm, Swed.). Cancer Metastasis Rev., 15(2), 241-245 (English) 1996. CODEN: CMRED4. ISSN: 0167-7659.
AB A review with 39 refs., with emphasis on factors and mechanisms involved in apoptosis, switching to angiogenic phenotype during tumor progression, and **angiogenesis** and metastasis. A deeper understanding of the mechanisms behind human tumor dormancy is of importance, as it could lead to the development of new therapies to extend the period of tumor quiescence.

L70 ANSWER 7 OF 15 BIOSIS COPYRIGHT 2000 BIOSIS
1996:397748 Document No.: PREV199699120104. Potential therapies of dormant metastases. **Holmgren, Lars (1)**; O'Reilly, Michael; Folkman, Judah. (1) Microbiology Tumor Biology Dep., Karolinska Inst., Stockholm Sweden. European Journal of Clinical Investigation, (1996) Vol. 26, No. SUPPL. 1, pp. A39. Meeting Info.: 30th Annual Scientific Meeting of the European Society for Clinical Investigation and the Medical Research Society of Great Britain Interlaken, Switzerland 1996 ISSN: 0014-2972. Language: English.

L70 ANSWER 8 OF 15 MEDLINE DUPLICATE 6
96071395 Document Number: 96071395. Dormancy of micrometastases: balanced proliferation and apoptosis in the presence of **angiogenesis** suppression [see comments]. **Holmgren L**; O'Reilly M S; Folkman J. (Department of Surgery, Children's Hospital, Boston, Massachusetts, USA.)NATURE MEDICINE, (1995 Feb) 1 (2) 149-53. Journal code: CG5. ISSN: 1078-8956. Pub. country: United States. Language: English.
AB In cancer patients, dormant micrometastases are often asymptomatic and clinically undetectable, for months or years, until relapse. We have studied dormant lung metastases under **angiogenesis** suppression in mice. The metastases exhibited rapid growth when the inhibition of **angiogenesis** was removed. Tumour cell proliferation, as measured by bromodeoxyuridine incorporation and immunohistochemical staining proliferating cell nuclear antigen, was not significantly different in dormant and growing metastases. However, tumour cells of dormant metastases exhibited a more than threefold higher incidence of apoptosis. These data show that metastases remain dormant when tumour cell proliferation is balanced by an equivalent rate of cell death and suggest that **angiogenesis** inhibitors control metastatic growth by indirectly increasing apoptosis in tumour cells.

L70 ANSWER 9 OF 15 BIOSIS COPYRIGHT 2000 BIOSIS
1996:52524 Document No.: PREV199698624659. Endogenous **angiogenesis** inhibitors. O'Reilly, Michael S.; **Holmgren, Lars**; Folkman, Judah. Children's Hosp., Harvard Med. Sch., Boston, MA 02115 USA. Molecular Biology of the Cell, (1995) Vol. 6, No. SUPPL., pp. 116A. Meeting Info.: Thirty-fifth Annual Meeting of the American Society for Cell Biology Washington, D.C., USA December 9-13, 1995 ISSN: 1059-1524. Language: English.

L70 ANSWER 10 OF 15 BIOSIS COPYRIGHT 2000 BIOSIS
1995:151320 Document No.: PREV199598165620. Treatment of transgenic mice with a regimen of **angiogenesis** inhibitors impairs tumor development. Parangi, S. (1); O'Reilly, M. O.; Christofori, G. (1); **Holmgren, L.**; Folkman, J.; Hanahan, D. (1). (1) Univ. Calif. San Francisco, San Francisco, CA 94143 USA. Proceedings of the American Association for Cancer Research Annual Meeting, (1995) Vol. 36, No. 0, pp. 104. Meeting Info.: Eighty-sixth Annual Meeting of the American Association for Cancer Research Toronto, Ontario, Canada March 18-22, 1995 ISSN: 0197-016X. Language: English.

L70 ANSWER 11 OF 15 BIOSIS COPYRIGHT 2000 BIOSIS
1995:375497 Document No.: PREV199598389797. Angiostatin: A circulating endothelial cell inhibitor that suppresses **angiogenesis** and tumor growth. O'Reilly, M. S. (1); **Holmgren, L.**; Shing, Y.; Chen, C.; Rosenthal, R. A.; Cao, Y.; Moses, M.; Lane, W. S.; Sage, E. H.; Folkman, J.. (1) Children's Hosp., Boston, MA 02115 USA. COLD SPRING HARBOR LABORATORY.. Cold Spring Harbor Symposia on Quantitative Biology, (1994) Vol. 59, pp. 471-482. Cold Spring Harbor Symposia on Quantitative Biology; The molecular genetics of cancer. Publisher: Cold Spring Harbor Laboratory Press 10 Skyline Drive, Plainview, New York 11803, USA. Meeting Info.: 59th Symposium on Quantitative Biology Cold Spring Harbor, New York, USA June 1-8, 1994 ISSN: 0091-7451. ISBN: 0-87969-068-2 (paper), 0-87969-067-4 (cloth). Language: English.

L70 ANSWER 12 OF 15 MEDLINE DUPLICATE 7
96063116 Document Number: 96063116. Angiostatin: a circulating endothelial cell inhibitor that suppresses **angiogenesis** and tumor growth. O'Reilly M S; **Holmgren L**; Shing Y; Chen C; Rosenthal R A; Cao Y; Moses M; Lane W S; Sage E H; Folkman J. (Children's Hospital, Boston, Massachusetts, USA..)COLD SPRING HARBOR SYMPOSIA ON QUANTITATIVE BIOLOGY, (1994) 59 471-82. Journal code: DMT. ISSN: 0091-7451. Pub. country: United States. Language: English.

L70 ANSWER 13 OF 15 MEDLINE DUPLICATE 8
95042728 Document Number: 95042728. Angiostatin: a novel **angiogenesis** inhibitor that mediates the suppression of metastases by a Lewis lung carcinoma [see comments]. O'Reilly M S; **Holmgren L** ; Shing Y; Chen C; Rosenthal R A; Moses M; Lane W S; Cao Y; Sage E H; Folkman J. (Department of Surgery, Children's Hospital, Boston, Massachusetts..)CELL, (1994 Oct 21) 79 (2) 315-28. Journal code: CQ4. ISSN: 0092-8674. Pub. country: United States. Language: English.
AB The phenomenon of inhibition of tumor growth by tumor mass has been repeatedly studied, but without elucidation of a satisfactory mechanism. In our animal model, a primary tumor inhibits its remote metastases. After tumor removal, metastases neovascularize and grow. When the primary tumor is present, metastatic growth is suppressed by a circulating **angiogenesis** inhibitor. Serum and urine from tumor-bearing mice, but not from controls, specifically inhibit endothelial cell

proliferation. The activity copurifies with a 38 kDa plasminogen fragment that we have sequenced and named angiostatin. A corresponding fragment of human plasminogen has similar activity. Systemic administration of angiostatin, but not intact plasminogen, potently blocks neovascularization and growth of metastases. We here show that the inhibition of metastases by a primary mouse tumor is mediated, at least in part, by angiostatin.

L70 ANSWER 14 OF 15 CAPLUS COPYRIGHT 2000 ACS
1994:241201 Document No. 120:241201 Potential dual roles of PDGF-B during human placental blood vessel formation. **Holmgren, Lars** (Dep. Exp. Alcohol Drug Res., Karolinska Hosp., Stockholm, Swed.). Endothelium, 1(3), 167-71 (English) 1993. CODEN: ENDTE9. ISSN: 1062-3329.
AB Development of the human placenta is characterized by continuous **angiogenesis** which proceeds until term pregnancy which makes the placenta a unique model system for the studies of normal human **angiogenesis**. This report compares the patterns of PDGF-B and PDGF .beta.-receptor protein expression in microcapillaries to those of larger blood vessels. Endothelial cells of microcapillaries co-express PDGF-B and PDGF .beta.-receptor protein as analyzed by immunohistochem. staining of frozen sections of term placenta. Endothelial cells of larger vessels expressed the PDGF-B protein whereas PDGF .beta.-receptor protein was not detectable. Pos. staining with receptor antibodies was detected in the surrounding pericytes and smooth muscle cells. These data support the hypothesis that capillary endothelial cells proliferate via a PDGF-mediated autocrine loop. Endothelial cells situated in larger vessels, may stimulate growth of the vessel intima by paracrine interactions.

L70 ANSWER 15 OF 15 MEDLINE DUPLICATE 9
92339355 Document Number: 92339355. **Angiogenesis** during human extraembryonic development involves the spatiotemporal control of PDGF ligand and receptor gene expression. **Holmgren L**; Glaser A; Pfeifer-Ohlsson S; Ohlsson R. (Laboratory for Molecular Development and Tumour Biology, Karolinska Hospital, Stockholm, Sweden..)DEVELOPMENT, (1991 Nov) 113 (3) 749-54. Journal code: ECW. ISSN: 0950-1991. Pub. country: ENGLAND: United Kingdom. Language: English.
AB We have examined the role of platelet-derived growth factor (PDGF) ligand and receptor genes in the angiogenic process of the developing human placenta. In situ hybridization analysis of first trimester placentae showed that most microcapillary endothelial cells coexpress the PDGF-B and PDGF beta-receptor genes. This observation indicates that PDGF-B may participate in placental **angiogenesis** by forming autostimulatory loops in capillary endothelial cells to promote cell proliferation. Endothelial cells of macro blood vessels maintained high PDGF-B expression, whereas PDGF beta-receptor mRNA was not detectable. In contrast, PDGF beta-receptor mRNA was readily detectable in fibroblast-like cells and smooth muscle cells in the surrounding intima of intermediate and macro blood vessels. Taken together, these data suggest that the PDGF-B signalling pathway appears to switch from an autocrine to a paracrine mechanism to stimulate growth of surrounding PDGF beta-receptor-positive mesenchymal stromal cells. Smooth muscle cells of the blood vessel intima also expressed the PDGF-A gene, the protein product of which is presumably targeted to the fibroblast-like cells of the mesenchymal stroma as these cells were the only ones expressing the PDGF alpha-receptor. PDGF-A expression was also detected in columnar

cytotrophoblasts where it may have a potential role in stimulating mesenchymal cell growth at the base of the growing placental villi. We discuss the possibility that the regulation of the PDGF-B and beta-receptor gene expression might represent the potential targets for primary angiogenic factors.